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Vitellogenin dynamics and reproductive morphology at sexual maturity of Philippine Mallard (*Anas platyrhynchos domesticus* L) fed with zinc supplemented diet

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Abstract. The vitellogenin (Vtg) is a precursor for the synthesis of egg yolk protein. Feeding with zinc-supplemented diet is hypothesized to increase the circulating Vtg thereby enhancing reproductive development. A total of 80 ducks, aged 16 weeks old, were randomly assigned to treatment groups; namely: group A with 40 ducks fed with 30 ppm zinc-supplemented diet (zinc positive) and group B with 40 ducks fed with no added zinc (zinc negative). The circulating Vtg at sexual maturity (155.11±10.83 days old) were determined from the blood sera. The sera were assayed for Vtg in duplicate using 96–well microplate and read the optical density at 415nm. The zinc concentration of the samples was calculated using the nonlinear regression $\Delta OD = a \times [Zn^{2+}] / (b + [Zn^{2+}]$. Results show that the circulating Vtg in the blood sera of ducks at sexual maturity were 0.69±0.07 µg Zn dL^{-1}. The feeding of zinc-treated diet had no significant influenced on the concentration of circulating Vtg. There was also insignificant difference in the reproductive morphology of ducks fed with or without added zinc. The Vtg concentration had no correlation with reproductive parameters but found to be positively correlated with liver weight (ρ=0.21) and negatively correlated with body weight (ρ = -0.24).
Keywords: Egg production, Reproductive morphology,Vitellogenin, Zinc.

Introduction

The egg production traits are governed by genetics, environment and interaction effects which can be regulated and modified under the current state of technological advances. Various methods are available from use of fundamental knowledge in breeding to the application of molecular technology. All these methods, however, require large population size, large facilities for research, organize systems for handling and evaluation of large data sets and are time-consuming. Hence, it is indeed vital that a research procedure be developed at least cost within relatively short period of time. The recent novel procedure is the association of vitellogenin (Vtg) with the expression of reproductive phenotype.

Vtg is a precursor molecule of the two major egg yolk phosphoproteins, namely: lipovitellin and phosvitin (Deeley *et al*, 1975). Vtg is synthesized in the liver and transported to the ovary where it is deposited in the oocyte. The association of Vtg and its receptors with reproductive phenotype has been demonstrated in zebra finch, *Taeniopygia guttata* (a songbird) and greater scaup, order: Anseriformes, *Aythya marila* (Han *et al*, 2009; Gorman *et al*, 2009).

The circulating Vtg can be assessed based on quantitative analysis of zinc concentration (µg Zn dL^{-1}) in the blood. The zinc concentration in serum/plasma labeled as vitellogenic zinc is a surrogate measure of circulating Vtg based on analysis that the Vtg is a zinc protein with 1 g atom zinc/220 kDa monomer (Montorzi *et al*, 1994). Specifically, Vtg zinc is present in lipovitellin in amounts equal to 1 mol of zinc/141 kDa (Montorzi *et al*, 1995).

The feeding of zinc-supplemented diet is hypothesized to increase the circulating Vtg thereby enhancing reproductive development in Philippine mallard (*Anas platyrhynchos domesticus* L).

Materials and Methods

Experimental Design

A total of 80 ducks, aged 16 weeks old, were randomly selected to determine vitellogenic zinc concentration in blood sera and measure the reproductive parameters at sexual maturity. All ducks were kept individually in a cage with identification code for recording purposes. Forty ducks were fed ration supplemented with 30 ppm dietary Zinc (zinc

positive, Sahin *et al*, 2002) using feed grade zinc oxide (MW = 81.41 g/mol) from 16 weeks of age until sexual maturity. The other 40 ducks were fed the control ration (zinc negative). To measure the reproductive parameters, 20 ducks each treatment was randomly selected and processed at sexual maturity.

Collection of Serum Sample

One milliliter blood samples were collected from each duck at sexual maturity. The blood samples were placed in a 1.5mL microtube and stored in ice chest before transporting to the laboratory. The coagulated blood samples were centrifuged at 1200 rpm for 10 min. About 200µL serum samples were collected from each blood sample. The serum samples were placed in a properly labeled 1.5mL microtube and stored at -20^{o}C until vitellogenic zinc assay.

Vitellogenic zinc concentration

A total of 80 sera were assayed in duplicate for vitellogenic zinc (Zn; zinc kit, BioAssay System, USA) as surrogate of vitellogenin following the described protocol using the 96–well microplate. Briefly, 50µL serum was transferred into wells where 200 µL working reagent was added. The mixtures were incubated for 30 min at room temperature and read the optical density (OD) at 415nm using the microplate reader (Model 680, S/N 123669). The OD reading was used in calculating the concentration of vitellogenic zinc in a serum sample. The zinc concentration of the samples were determined from the standard values by non-linear regression using the formula $\Delta OD = a \times [Zn^{2+}] / (b + [Zn^{2+}]$. Calculation and graphical formatting were performed in Microsoft Excel. The vitellogenic zinc concentration was expressed in µg/dL using the conversion factor 1µM = 6.5µg/dL.

Measurement of reproductive morphology

The weight of freshly collected ovary (OVA), oviduct (OVI), stroma, and liver (LW) were determined from 20 ducks each treatment. All units of weight were expressed in grams. The ovarian follicles were counted and categorized as large yellow follicles (>10mm) and small yellow follicles (5–10 mm). The post-ovulatory follicles were also counted. The gonadosomatic index was derived from the weights of OVA while the oviductosomatic index was derived from the weights of OVI in proportion to BW. The hepatosomatic index was derived from the LW in proportion to BW. All units of indices were expressed in percentage.

Results and Discussion

Vitellogenin Dynamics

Similarity in vitellogenin concentrations (VTG, µg Zn dL^{-1}) at sexual maturity was detected in the blood sera of ducks fed with or without 30 ppm zinc (Zn) as zinc oxide starting at 16 weeks of age ($p>\alpha$). This shows that the supplemental zinc in the ration did not induce a significant change in the concentration of circulating Vtg. It has been observed, however, there was significant increase in zinc concentration in the kidney and liver of 66-week old hens fed 1% Zn as Zn propionate and Zn acetate (Park *et al*, 2004). Similarly, the 30 ppm Zn has produced positive effect on egg production based on egg weight, measures of egg quality, and feed efficiency (Sahin *et al*, 2002).

The Vtg concentration in blood sera of ducks at sexual maturity was 0.69±0.07 µg Zn dL^{-1} which is similar with Vtg concentration at 17 to 22 weeks of age (0.65±0.10 µg Zn dL^{-1}, Study 1) but significantly lower than the Vtg at 40 weeks of age (1.80±1.47 µg Zn dL^{-1}, Study 3). The changes in Vtg concentration during these three reproductive periods was a cubic function at R^2 = 30.52% indicating that 69.48% of the total variation is attributed to other variables not shown in the regression. This is consistent with the previous observation that age signifies only the timing of Vtg production and Vtg demand from the developing ovarian follicle hierarchy (Gorman *et al*, 2009; Salvante and Williams, 2002). This observation was demonstrated in female greater scaup (order: Anseriformes, *Aythya marila*), which shows that Vtg was lower with non-developed ovaries (0.58±0.05 mg Zn mL^{-1}) than with full ovarian follicle hierarchy (3.38±0.40 mg Zn mL^{-1}) (Gorman *et al*, 2009). In female zebra finches (*Taeniopygia guttata*), Vtg was detectable only during yolk development remaining high (1.43–1.82 µg Zn ml^{-1}) through to the 3-egg stage, then declined at the 5-egg stage (0.78±0.32 µg Zn ml^{-1}) and were undetectable at clutch completion (Salvante and Williams, 2002).

Variance component analysis shows that all variations (100%) in Vtg is attributed to individual effects indicating wide differences in hepatic production and demand from the developing ovarian follicle hierarchy (cv=15.43%). A wide inter-individual variation in plasma Vtg (0.47–4.26 μg Zn ml^{-1}) was observed also in female zebra finches at the 1-egg stage (Salvante and Williams, 2002). High intra-individual repeatability (r=0.87–0.93) of plasma Vtg was also identified (Salvante and Williams, 2002).

Age and body weight

The age at sexual maturity was 155.11±10.83 d (Table 1). There was relatively high level of uniformity at sexual maturity (CV = 8.88%). Ducks have reached sexual maturity at statistically similar age as those in Study 1 (153.35±11.79d) and Study 3 (156.45± 15.68d).

The body weight at sexual maturity was 1.50±0.14 kg ranges from 1.17 to 1.79 kg at 95% confidence limit. This value is statistically similar to BW at same age of ducks in Study 1 (1.47±0.16) and Study 3 (1.48±0.16 kg). The variation in BW is attributed to inter-individual differences (100%).

Ovary weight

The ovary weights of ducks at sexual maturity were 48.44±13.42g which are heavier than the ovary weights at 17 to 22 weeks of age (1.85±0.97g, Study 1). There was relatively low difference in the ovary weight (CV=27.72%). The weight of the ovary had increased during sexual maturity because of the growing follicle hierarchy. In *Gallus*, vitellogenesis is generally recognized as occurring in three distinct phases namely; *a) initial period of slow-growth which can be months or years in duration, b) intermediate-growth phase of about 60 days duration, and c) rapid-growth phase, characterized by extensive yolk deposition and the first meiotic reduction division*. The follicular growth progresses to approximately four millimeters in diameter during the intermediate phase (Van Krey, 1990).

Large yellow follicles

The number of large yellow follicles at sexual maturity was 6.95±1.75 with a CV of 24.85%. This conforms to chickens subjected to photostimulation which had 6.72 LYF and chickens selected for early maturity (7.0 LYF) and late maturity (6.4 LYF) (Renema *et al*., 2001; Robinson *et al*., 2001).

Small yellow follicles and Postovulatory follicles

The number of small yellow follicles at sexual maturity was 3.98±3.45 with a CV of 87.88%. The finding had fewer SYF than in chickens selected for early maturity (5 SYF) and late maturity (5.2 SYF) (Robinson *et al*., 2001).

The number of postovulatory follicles at sexual maturity was 2.03±0.15 with a CV of 7.81%. The number of postovulatory follicles was relatively similar to chickens selected for early maturity (2.4 POF) and late maturity (2.5 POF) (Robinson *et al*., 2001).

Clutch size

The clutch size at sexual maturity was 10.93±4.09 at a CV of 37.80%. The clutch size observed was smaller than in chickens selected for early maturity and late maturity having a clutch size of 12 and 11.6, respectively (Robinson *et al*., 2001).

Table 1. Vitellogenin and reproductive parameters at sexual maturity of Philippine Mallard (*Anas platyrhynchos domesticus* L).

Parematers	n	MEAN ± SD
Vitellogenin (µg Zn dL^{-1})	80	0.69±0.07
Age at first egg, d	80	155.11±10.83
Body weight at first egg, kg	80	1.50±0.14
First egg weight, g	80	49.78±7.78
Normal shelled eggs (1st egg), %	80	100
Liver weight, g	40	38.34±4.41
Ovary weight, g	40	48.44±13.42
Oviduct weight, g	40	49.15±6.80
Stroma weight, g	40	5.63±1.60
Hepatosomatic index, %	40	26.06±2.63
Gonadosomatic index, %	40	33.08±9.57
Oviductosomatic index, %	40	33.36±3.82
Number of large yellow follicles	40	6.95±1.75
Number of small yellow follicles	40	3.98±3.45
Number of post-ovulatory follicles	40	2.03±0.15
Clutch size	40	10.93±4.09

Relationship of Vitellogenin with Laying Traits

Significant relationship of vitellogenin concentration (Vtg, µg Zn dL^{-1}) at sexual maturity with selected parameters of reproduction and laying traits was not observed. This is unusual when compared to other observations showing significant relationship with body weight, liver weight (Study 1), age at first egg, laying duration, hen-day rate egg production, pause length, and body weight at 40 week of age (Study 3). This is also contrary to findings that demonstrated a positive correlation of liver weight and Vtg levels (Christians and Williams, 1999). In laying hens, approximately 50% of the liver's daily protein synthesis is attributed to Vtg production, potentially tripling the amount of protein in circulation (Gruber, 1972 in Vézina *et al*., 2003).

On the other hand, previous studies had reported that Vtg is correlated with ovary mass and egg weight. The Vtg concentrations were found to increase rapidly in association with small increases in ovary mass during rapid follicle growth (Gorman e al., 2009). *However, there was some evidence for mismatching of supply and demand: (a) precursor concentrations increased throughout the laying cycle even though the number of developing follicles decreased.* This condition happens *because of a requirement to maintain a large precursor pool to maintain high uptake rates; and (b) in birds with a full follicle hierarchy, precursor concentrations were negatively correlated with total follicle mass. This suggests that high uptake rates in large follicles can actually deplete circulating precursor concentrations* (Challenger *et al*., 2001).

A diet-dependent relationship between Vtg and egg size was identified also in zebra finches (*Taeniopygia guttata*), which shows that low plasma Vtg levels were associated with both very small (<0.90 g) and very large (>1.15 g) egg sizes (Salvante and Williams, 2002). Moreover, variation in ovary mRNA expression was correlated with inter-individual variation in clutch size and laying interval while variation in the third largest follicle VTG/VLDL-R mRNA expression was correlated with inter-individual variation in egg mass and largest follicle mass (Han *et al*, 2009).

Conclusions

The supplementation of 30 ppm zinc (Zn) as zinc oxide in the diet of Philippine mallard (*Anas platyrhynchos domesticus* L) from 16 weeks of age until sexual maturity did not induce significant change in the circulating Vtg. There was relatively low difference in the ovary weight (CV=27.72%). The number of large yellow follicles at sexual maturity was 6.95±1.75 (CV=24.85%) while the small yellow follicles were 3.98±3.45 (CV=87.88%). The number of postovulatory follicles at sexual maturity was 2.03±0.15 (CV=7.81%).

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References

Deeley R.G., Mullinix K.P., Wetekam W., Kronenberg H.M., Meyers M., Eldridge J.D., Goldberger R.F.. 1975. Vitellogenin synthesis in avian liver: vitellogenin is precursor of egg-yolk phosphoproteins. Journal of Biological Chemistry, 250:9060–9066.

Gorman K.B., Esler D., Walzem R.L., Williams T.D. 2009. Plasma yolk precursor dynamics during egg production by female Greater scaup (A*ythya marila*): characterization and indices of reproductive state. Physiological and Biochemical Zoology, 82(4):372–381.

Han D., Haunerland N.H., Williams T.D. 2009. Variation in yolk precursor receptor mRNA expression is a key determinant of reproductive phenotype in the zebra finch (*Taeniopygia guttata*). The Journal of Experimental Biology, 212: 1277-1283.

Mitchell M.A., Carlisle A.J. 1991. Plasma zinc as an index of vitellogenin production and reproductive status in the domestic fowl. Comparative Biochemistry and Physiology, 100A: 719–724.

Montorzi M., Falchuk K.H., Vallee B.L. 1994. *Xenopus laevis* vitellogenin is a zinc protein. Biochemical and Biophysical Research Communication, 200(3):1407-1413.

Montorzi M., Falchuk K.H., Vallee B.L. 1995. Vitellogenin and lipovitellin: zinc proteins of *Xenopus laevis* oocytes. Biochemistry, 34(34):10851-10858.

Park S.Y., Birkhold S.G., Kubena L.F., Nisbet D.J., Ricke S.C. 2004. Effects of high zinc diets using zinc propionate on molt induction, organs, and postmolt egg production and quality in laying hens. Poultry Science. 83:24–33.

Sahin N., Onderci M., Sahin K. 2002. Effects of dietary chromium and zinc on egg production, egg quality, and some blood metabolites of laying hens reared under low ambient temperature. Biological Trace Element Research. 85(1):47-58.

Salvante K.G., Williams T.D. 2002. Vitellogenin dynamics during egg-laying: daily variation, repeatability and relationship with egg size. Journal of Avian Biology, 33: 391-398.

Van Krey H.P. 1990. Reproductive biology in relation to breeding and genetics. In: Crawford R.D. (ed) Poultry Breeding & Genetics. Developments in Animal and Veterinary Sciences, 22. Elsevier. 61-90.